

019 deletion (GGCACCTCCCCTTAAC) (SEQ ID NO:42) and a 4 nucleotide deletion (TGCA) 6 nucleotides upstream (Figure 8B). These observations indicate that the t(1;14)(q21;q32) in FR4 cells represents a balanced reciprocal translocation possibly facilitated by the presence of homologous sequences (CTTAAC) on both chromosomes.

REMARKS

In the April 9, 2003 Communication, the Examiner states that the subject application fails to comply with the requirements of 37 C.F.R. §1.821-1.825. Specifically, the Examiner states that throughout the specification of the subject application, polynucleotides and polypeptides of IRTA are recited without referring to the corresponding SEQ ID NOs of the submitted Sequence Listing.

In response, applicant has hereinabove amended the specification to properly identify the sequences by SEQ ID NO where appropriate. A marked-up version of the amended paragraphs is annexed hereto as **Exhibit A**. Applicant maintains that the amendments to the specification do not constitute new matter. Accordingly, applicant respectfully requests that this Amendment be entered.

In view of this Amendment, applicant maintains that the subject application satisfies the requirements of 37 C.F.R. §1.821-1.825.


If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's

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undersigned attorneys invite the Examiner to telephone them at the number provided below.

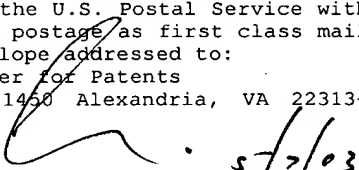
No fee is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:
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Alan J. Morrison
Reg. No. 37,399

5/7/03

Date

Marked-up copy of the amendments to the specification

The paragraph starting on page 6, line 26 has been amended as follows:

This invention provides a method for detecting a B cell malignancy or a type of B cell malignancy in a sample from a subject wherein the B cell malignancy comprises a 1q21 chromosomal rearrangement which comprises: a) obtaining RNA from the sample from the subject; b) contacting the RNA of step (a) with a nucleic acid molecule of at least 15 contiguous nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of an isolated RNA encoding human IRTA protein selected from the group consisting of human IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9), under conditions permitting hybridization of the RNA of step (a) with the nucleic acid molecule capable of specifically hybridizing with a unique sequence included within the sequence of an isolated RNA encoding human IRTA protein, wherein the nucleic acid molecule is labeled with a detectable marker; and c) detecting any hybridization in step (b), wherein [detecion] detection of hybridization indicates presence of B cell malignancy or a type of B cell malignancy in the sample.

The paragraph starting on page 8, line 9 has been amended as follows:

This invention provides an antibody/antibodies directed to an epitope of a purified IRTA1, IRTA2, IRTA3, IRTA4 or IRTA5 protein, or fragment(s) thereof, having the amino

acid sequence set forth in any of Figures 18A (SEQ ID NO:1), 18B-1-18B-3 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), 18C-1-18C-2 (SEQ ID NO:5), 18D-1-18D-2 (SEQ ID NO:7) or 18E-1-18E-2 (SEQ ID NO:9), respectively.

The paragraph starting on page 8, line 15 has been amended as follows:

This invention provides an antibody directed to a purified IRTA protein selected from the group consisting of IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9).

The paragraph starting on page 8, line 19 has been amended as follows:

This invention provides a pharmaceutical composition comprising an amount of the antibody directed to an IRTA protein effective to bind to cancer cells expressing an IRTA protein selected from the group consisting of human IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9), so as to prevent growth of the cancer cells and a pharmaceutically acceptable carrier.

The paragraph starting on page 9, line 1 has been amended as follows:

This invention provides a method of diagnosing B cell malignancy which comprises a 1q21 chromosomal rearrangement in a sample from a subject which comprises:
a) obtaining the sample from the subject; b) contacting the sample of step (a) with an antibody directed to a

purified IRTA protein capable of specifically binding with a human IRTA protein selected from the group consisting of human IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9) IRTA protein on a cell surface of a cancer cell under conditions permitting binding of the antibody with human IRTA protein on the cell surface of the cancer cell, wherein the antibody is labeled with a detectable marker; and c) detecting any binding in step (b), wherein [~~detecion~~] detection of binding indicates a diagnosis of B cell malignancy in the sample.

The paragraph starting on page 29, line 20 has been amended as follows:

Nucleic acid molecules encoding proteins designate "MUM-2" (SEQ ID NO:15) and "MUM-3" (SEQ ID NO:17, SEQ ID NO:19 or SEQ ID NO:4) proteins in the First Series of Experiments are now called "IRTA-1" and "IRTA-2" genes, i.e. nucleic acid molecules which encode IRTA-1 (SEQ ID NO:1) and IRTA-2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41) proteins respectively. IRTA-3 (SEQ ID NO:5), -4 (SEQ ID NO:7) and -5 (SEQ ID NO:9) proteins are members of the same the immunoglobulin gene superfamily as are the IRTA-1 and IRTA-2 proteins.

The paragraph starting on page 32, line 4 has been amended as follows:

This invention provides a method of producing an IRTA polypeptide (protein) which comprises: (a) introducing a vector comprising an isolated nucleic acid which encodes an immunoglobulin receptor, Immunoglobulin superfamily

Receptor Translocation Associated, IRTA, protein into a suitable host cell; and (b) culturing the resulting cell so as to produce the polypeptide. In further embodiments, the IRTA protein produced by the above-described method may be recovered and in a still further embodiment, may be purified either wholly or partially. In an embodiment the IRTA protein may be any of IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9) protein. In further embodiments, any of the IRTA proteins may be mammalian proteins. In still further [embodiments] embodiments, the mammalian proteins may be human or mouse IRTA proteins.

The paragraph starting on page 32, line 20 has been amended as follows:

IRTA genes, nucleic acid molecules encoding IRTA [proteins] proteins IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9), are useful for the production of the IRTA proteins encoded thereby. IRTA proteins are useful for production of antibodies; such antibodies are used as reagents for differential diagnosis of lymphoma subtypes in hematopathology. Antibodies directed against IRTA proteins and which bind specifically to IRTA proteins also have therapeutic uses, i.e. to specifically target tumor cells, which may be used and administered similarly to "Rituximab" (an anti-CD20 antibody), which is an antibody approved by the FDA for therapy of relapsed CD20-positive lymphomas (Foon K., Cancer J. 6(5):273). Anti-IRTA1, anti-IRTA2, anti-IRTA3, anti-IRTA4. and anti-IRTA5 antibodies are also useful markers for isolation of specific subsets of B cells in

research studies of normal and tumor B cell biology. Moreover, anti-IRTA1, anti-IRTA2, anti-IRTA3, anti-IRTA4 and anti-IRTA5 antibodies are useful research reagents to experimentally study the biology of signaling in normal and tumor B cells.

The paragraph starting on page 33, line 9 has been amended as follows:

Methods of introducing nucleic acid molecules into cells are well known to those of skill in the art. Such methods include, for example, the use of viral vectors and calcium phosphate co-precipitation. Accordingly, nucleic acid molecules encoding IRTA proteins IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9) may be introduced into cells for the production of these IRTA proteins.

The paragraph starting on page 35, line 7 has been amended as follows:

This invention provides an isolated nucleic acid molecule comprising at least 15 contiguous nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of the isolated nucleic acid molecule encoding IRTA protein. In an embodiment, the IRTA protein may be IRTA1, IRTA2, IRTA3, IRTA4 or IRTA5 protein, or fragment(s) thereof, having the amino acid sequence set forth in any of Figures 18A (SEQ ID NO:1), 18B-1-18B-3 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), 18C-1-18C-2 (SEQ ID NO:5), 18D-1-18D-2 (SEQ ID NO:7) or 18E-1-18E-2 (SEQ ID NO:9), respectively. In other embodiments, the isolated nucleic acid molecules are

labeled with a detectable marker. In still other embodiments of the isolated nucleic acid molecules, the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

The paragraph starting on page 37, line 28 has been amended as follows:

In preferred embodiments of the antisense oligonucleotide, the ITRA protein selected from the group consisting of human IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9) protein. In further embodiments of any of the above-described oligonucleotides of nucleic acid molecules encoding the IRTA1, IRTA2, IRTA3, IRTA4 and/or IRTA5 proteins, the nucleic acid may be genomic DNA or cDNA.

The paragraph starting on page 38, line 15 has been amended as follows:

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. As used herein, a "unique sequence" is a sequence specific to only the nucleic acid molecules encoding the IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9) proteins. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length

and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid molecules encoding the IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9) proteins is useful as a diagnostic test for any disease process in which levels of expression of the corresponding IRTA1, IRTA2, IRTA3, IRTA4 and/or IRTA5 proteins is altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes mammalian IRTA1, IRTA2, IRTA3, IRTA4 and/or IRTA5 proteins or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed herein), electrophoresed, and cut out of the resulting gel. The oligonucleotide probes are useful for 'in situ' hybridization or in order to locate tissues which express this IRTA gene family, and for other hybridization assays for the presence of these genes (nucleic acid molecules encoding any of the IRTA1-IRTA5 [~~protiens~~] proteins) or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes an IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) or IRTA5 (SEQ ID NO:9) protein are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by

the use of amplification techniques such as the Polymerase Chain Reaction.

The paragraph starting on page 45, line 4 has been amended as follows:

This invention provides an antibody directed to a purified IRTA protein selected from the group consisting of IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9). In a preferred embodiment of the anti-IRTA antibody the IRTA protein is human IRTA protein. The IRTA protein may be any mammalian IRTA protein, including a murine IRTA protein. In a further embodiment of any the above-described antibodies, the antibody is a monoclonal antibody. In another embodiment, the monoclonal antibody is a murine monoclonal antibody or a humanized monoclonal antibody. As used herein, "humanized" means an antibody having characteristics of a human antibody, such antibody being non-naturally occurring, but created using hybridoma techniques wherein the antibody is of human origin except for the antigen determinant portion, which is murine. In yet another embodiment, the antibody is a [polyoclonal] polyclonal antibody.

The paragraph starting on page 46, line 8 has been amended as follows:

This invention provides a pharmaceutical composition comprising an amount of the antibody directed to an IRTA protein effective to bind to cancer cells expressing an IRTA protein selected from the group consisting of human IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or

SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9) so as to prevent growth of the cancer cells and a pharmaceutically acceptable carrier. The anti-IRTA antibody may be directed to an epitope of an IRTA protein selected from the group consisting of IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9). The IRTA proteins may be human or mouse IRTA proteins.

The paragraph starting on page 48, line 4 has been amended as follows:

In an embodiment of the above-described method of diagnosing B cell malignancy, the IRTA protein is selected from the group consisting of IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9). In another embodiment of the method the IRTA protein is human or mouse IRTA protein. In a further embodiment IRTA protein is purified. In a preferred embodiment of this method, the B cell malignancy is selected from the group consisting of B cell lymphoma, multiple myeloma, Burkitt's lymphoma, marginal zone lymphoma, diffuse large cell lymphoma and follicular lymphoma. In yet another embodiment of this method, the B cell lymphoma is Mucosa-Associated-Lymphoid Tissue B cell lymphoma (MALT). In another preferred embodiment of this method, the B cell lymphoma is non-Hodgkin's lymphoma.

The paragraph starting on page 49, line 19 has been amended as follows:

This invention provides a method of detecting human IRTA protein in a sample which comprises: a) contacting the sample with any of any of the above-described anti-IRTA antibodies under conditions permitting the formation of a complex between the antibody and the IRTA in the sample; and b) detecting the complex formed in step (a), thereby detecting the presence of human IRTA in the sample. In an [embodiement] embodiment the IRTA protein detected may be an IRTA1, IRTA2, IRTA3, IRTA4 or IRTA5 protein, having an amino acid sequence set forth in any of Figures 18A (SEQ ID NO:1), 18B-1-18B-3 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), 18C-1-18C-2 (SEQ ID NO:5), 18D-1-18D-2 (SEQ ID NO:7) or 18E-[e]1-18E-2 (SEQ ID NO:9). As described hereinabove detection of the complex formed may be achieved by using antibody labeled with a detectable marker and determining presence of labeled complex. Detecting human IRTA protein in a sample from a subject is another method of diagnosing B cell malignancy in a subject. In an embodiment of this method of diagnosis, the B cell malignancy is selected from the group consisting of B cell lymphoma, multiple myeloma, Burkitt's lymphoma, marginal zone lymphoma, diffuse large cell lymphoma and follicular lymphoma. In yet another embodiment of this method, the B cell lymphoma is Mucosa-Associated-Lymphoid Tissue B cell lymphoma (MALT). In another preferred embodiment of this method, the B cell lymphoma is non-Hodgkin's lymphoma.

The paragraph starting on page 50, line 22 has been amended as follows:

This invention provides a method of treating a subject having a B cell cancer which comprises administering to the subject an amount of anti-IRTA antibody effective to

bind to cancer cells expressing an IRTA protein so as to prevent growth of the cancer cells and a pharmaceutically acceptable carrier, thereby treating the subject. Growth and proliferation of the cancer cells is thereby inhibited [amd] and the cancer cells die. In an embodiment of the above-described method, the IRTA protein is selected from the group consisting of human IRTA1 (SEQ ID NO:1), IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41), IRTA3 (SEQ ID NO:5), IRTA4 (SEQ ID NO:7) and IRTA5 (SEQ ID NO:9). In a preferred embodiment of the above-described method of treating a subject having a B cell cancer, the anti-IRTA antibody is a monoclonal antibody. In another embodiment of the method, the monoclonal antibody is a murine monoclonal antibody or a humanized monoclonal antibody. The antibody may be a chimeric antibody. In a further embodiment, the anti-IRTA antibody is a polyclonal antibody. In an embodiment, the polyclonal antibody may be a murine or human polyclonal antibody. In a preferred embodiment, the B cell cancer is selected from the group consisting of B cell lymphoma, multiple myeloma, Burkitt's lymphoma, mantle cell lymphoma marginal zone lymphoma, diffuse large cell lymphoma and follicular lymphoma. In another preferred embodiment, the B cell lymphoma is Mucosa-Associated-Lymphoid Tissue B cell lymphoma (MALT). In a further preferred embodiment, the B cell lymphoma is non Hodgkin's lymphoma. In a preferred embodiment of the above-described method of treating a subject having a B cell cancer, administration of the amount of anti-IRTA antibody effective to bind to cancer cells expressing an IRTA protein is intravenous, intraperitoneal, intrathecal, intralymphatic, intramuscular, intralesional, parenteral, epidural, subcutaneous; by infusion, liposome-mediated delivery, aerosol delivery;

topical, oral, nasal, anal, ocular or otic delivery. In another preferred embodiment of the above-described methods, the anti-IRTA antibody may be conjugated to a therapeutic agent. In further preferred embodiments, the therapeutic agent is a radioisotope, toxin, toxoid, or chemotherapeutic agent.

The paragraph starting on page 61, line 5 has been amended as follows:

Digoxigenin-containing antisense and sense cRNA probes were transcribed with T3 and T7 RNA polymerase, respectively, from linearized pBluescript KS+ plasmids containing coding region of cDNAs, [()nucleotides 62 to 1681 of IRTA1 (SEQ ID NO:1) and 18 to 2996 of IRTA2 (SEQ ID NO:44, SEQ ID NO:3 or SEQ ID NO:41). Hyperplastic human tonsillar tissue surgically resected from children in Babies' Hospital, Columbia Presbyterian Medical Center was snap frozen in powdered dry ice. Cryostat sections were stored for several days at -80 degrees C prior to processing. Non-radioactive *in situ* hybridization was performed essentially as described (Frank et al., 1999), except that fixation time in 4% paraformaldehyde was increased to 20 minutes, and proteinase K treatment was omitted. The stringency of hybridization was 68 degrees C, in 5X SSC, 50% formamide. Alkaline phosphatase-conjugated anti-digoxigenin antibody staining was developed with BCIP/NBT substrate.

The paragraph starting on page 63, line 28 has been amended as follows:

Sequence analysis of the breakpoint regions on the derivative chromosomes and alignment with the germline

14q32 and 1q21 loci (SEQ ID NO:24 and SEQ ID NO:26, respectively) revealed that the breakpoint had occurred in the intron between the CH3 and the transmembrane exon of Ca_1 on chromosome 14. Although the breakpoint region was devoid of recombination signal sequences (RSS) or switch signal sequences (Kuppers et al., 1999), the sequence CTTAAC (underlined on Figure 8B) was present in both germline chromosomes 14 and 1 at the breakpoint junction (SEQ ID NO:25 and SEQ ID NO:27, respectively). One copy of this sequence was present in each of the derivative chromosomes, with a slight modification in the der(1) copy (point mutation in the last nucleotide: C to G). The nucleotides AT preceding CTTAAC on chromosome 1 were also present in both derivative chromosomes (Figure 8B). The translocation did not result in any loss of chromosome 1 sequences. On the other hand, in the chromosome 14 portion of der(1) we observed two deletions upstream of the breakpoint junction: a 16 nucleotide deletion (GGCACCTCCCCTTAAC) (SEQ ID NO:42) and a 4 nucleotide deletion (TGCA) 6 nucleotides upstream (Figure 8B). These observations indicate that the t(1;14)(q21;q32) in FR4 cells represents a balanced reciprocal translocation possibly facilitated by the presence of homologous sequences (CTTAAC) on both chromosomes.